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REMARKS

Entry of this amendment is respectfully requested. No new matter is added by the amendment, as the amended claims are fully supported by the application as filed.

Claims 61-112 were examined. Applicant has amended claim 61. Claims 113 and 114 are newly presented.

Rejections under 35 USC §112

The examiner has rejected claims 61-62, 64-76 and 99-112 under §112, first paragraph, as based on a disclosure which is not enabling. The examiner states that the method of incorporating enzymes into thermoplastic or thermosetting polymers and formulation in which mere wetting of device activates oxidase critical or essential to the practice of the invention, but not included in the claim(s) is not enabled by the disclosure. Also, the specification specifically states that room temperature vulcanization elastomers must be used in fabricating the device from hydrophobic polymers since enzymes will denature, but the claims require the use of thermoplastic or thermosetting polymers. The examiner goes on to state that the claims can also include as proton donor or oxidizing agent an oxidase, but that there does not appear to be any disclosure which shows how the oxidase can be incorporated in view of the prohibition against their use, as indicated above. The examiner indicates that with respect to glucose oxidase, the specification fluids must contain glucose and that even exposure to water does not release the oxidant. There does not appear to be a disclosure which shows how said oxidases can be activated by simply setting the device absent presence of the appropriate substrate in view of the above.

Claims 61-62, 64-76 and 99-112 stand rejected under §112, first paragraph, because the specification, while being enabling for the specific oxidant with the appropriate oxidizing agent, reducing agent and proton donating agent as disclosed, and the disclosed processes of fabricating the devices does not reasonably provide enablement for oxidants, oxidizing agents and reducing agents or proton donating agents, where the disclosure is silent as to the appropriate combination of said agents or devices made by other processes.

Claims 61-62, 64-76 and 99-112 stand rejected under §112, second paragraph, as being incomplete for omitting essential steps, such as omission amounting to a gap between the steps. These grounds of rejection are respectively traversed.

Attention is directed to the specification as follows:

Examples of substantial step by step detail can be found, for example, in describing the oxidases and their incorporation into the polymer materials as stated on:

p. 10, paragraph 5, line 2 – 3 (*"...or an enzyme acting on a substrate which catalyzes formation of an acid product"*);

p. 11, paragraph 3, line 3 – 10 (*"...an oxidizing or reducing agent, or a reactant which forms the oxidizing or reducing agent, or a proton source may be absent from the polymeric matrix, so that the oxidant producing component is stable until contacted by water and by the oxidizing or reducing agent, and/or proton source. For example, hydrogen peroxide as an oxidizing agent may be produced by a substrate oxidase enzyme in which one or both of the substrate and the enzyme is provided by the body fluid. Thus, device stability is achieved ..."*);

p. 11, paragraph 4, line 1 through p. 12, paragraph 1, line 7 (*"A variety of suitable polymers ...including ...hydrogels ...polyurethanes...polyacrylates ... celluloses and starch ...Particles of the oxidant producing component, and ...are typically added to the polymeric material (or to elastomers of a two component formulation ...processed ...molding or extruding into the medical device."*);

p. 13, paragraph 2 through p. 14, paragraph 1 wherein specific weights and units of enzyme activity are described in detail;

p. 14, paragraph 3 through p. 15, paragraph 3, starting on lines 1 -2 (*"...the oxidizing means is selected ...consisting of akali iodine oxide salts, peracids, H2O2-generating enzyme oxidases, and combinations thereof ..."*) and ending with detailed descriptions of iodide and enzyme concentrations incorporated into polymers materials (*"Preferably, the hydrogel agent is ...linear polyacrylates, cross-linked polyacrylates, hydroxyalkyl celluloses, ...chitosan polymers, salts of alginic acid ...Preferably, the concentration of iodide in the hydrogel device formulation is from ...0.1 mM to ...200 mM ...the ...oxidizing means is present ..from ..2 mg/ml to ...when the oxidizing means is a H2O2 generating enzyme oxidase ...330,000 IU per gram solid."*);

p. 17, paragraphs 2 and 3, p. 18, paragraphs 1 and 2, p. 20, paragraph 2, p. 29, paragraph 2, lines 7 – 11;

p. 31, paragraph 2, p. 32, paragraph 2 and p. 33, paragraph 2; p 39, paragraph 2; p. 40 – 41, in particular, wherein specificity of the devices is discussed (cf., p. 42, paragraph 1 – *"...formulation allows for the device to activate and lay down anti-infective activity only upon contact with body fluids containing glucose. Even exposure to water does not active the release of iodine."*).

Similar detailed step by step descriptions can be found concerning the use of iodide salts and combinations thereof absent the use of oxidases in the specifications of the application which all together leave no doubt as to how the invention can be made by those knowledgeable in the art.

Rejections under 35 USC §102 and §103

Claims 62-62, 64-66, 70, 99-109, 106 and 112 stand rejected under §102(b) as anticipated by or, in the alternative, under §103(a) as obvious over Trescony, et al. (US 5,994,444). These grounds of rejection are respectively traversed.

In one embodiment of the present invention, as set forth in claim 1, an implantable anti-infective medical device is selected from the group consisting of catheters, prostheses, shunts, stents, and leadwires, and comprises a solid polymeric matrix selected from the group selected from a two component elastomer formulation, room temperature vulcanization elastomers, thermoplastic polymers, thermosetting polymers, and hydrogels. containing within the matrix solid particles are in the matrix. The solid particles are an oxidant-producing component comprising an iodine-containing salt, a reducing agent consisting of an iodide salt or reactants provided by body fluids, and an oxidizing agent consisting of anhydrous alkali iodine oxide salts, inorganic and organic peracids, and combinations thereof, that when wetted, causes the formation of an oxidant and sustained release of the thus-formed oxidant into and about the polymeric matrix so that the matrix serves as an anti-infective reservoir.

Support for various elements in claim 1 is found as follows:

"...a solid polymeric matrix selected from the group consisting of a two component elastomer formulation..." page 12, paragraph 1);

"...room temperature vulcanization elastomers..." page 33, paragraph 3;

..."containing within the matrix solid particles of an oxidant-producing component comprising an iodine-containing salt..."page. 9, paragraph 4;

"...a reducing agent consisting of an iodide salt..."page 10, paragraph 1)

"... or reactants provided by body fluids..."paragraph 11, paragraph 3; and

"...an oxidizing agent consisting of anhydrous alkali iodine oxide salts, inorganic and organic peracids, and combinations thereof..." page 13 paragraph 1.

U.S. Patent No. 5,994,444 (Trescony, et al.,) is directed to making nitric oxide and not to medical devices, or polymers, incorporating solid reactants, anti-infective activity. Nitric oxide has no intrinsic antimicrobial activity. The only anti-infective activity of nitric oxide is very limited and is from oxidation of nitric oxide. See (see p. 185, column 2, lines 8-10 *in* Burgner, D., Rockett, K. and Kwiatkowski, D. (1999). *Nitric oxide and infectious diseases.* Arch Dis Child **81**: 158-185).

The mere recitation by Trescony, et al., of using acids and reductants fails to teach or suggest how to fabricate a device with anti-infective properties. Trescony, et al., does not teach nor suggest conference of anti-infective activity to its devices, and instead merely notes, that among reductants useful in making nitric oxide, one of the reductants that can be used is potassium iodide. See column 5, line 29-30, and column 6, lines 2-5.

In particular, the concentration of nitric oxide, and iodine, produced in Trescony, et al., is insufficient in providing anti-infective activity to a device. Trescony, et al., discloses that nitric oxide

"..is unstable at physiological O2 tensions....rapidly inactivated (in seconds) by oxyhemogolbin within red blood cells..." (column 1, lines 20 – 24).

Trescony, et al., further shows that nitric oxide is formed in ***picomole*** quantities, see Figures 1-4, and that nitric oxide production falls off to very much lower levels above a pH of 4. See Figure 3 and Example IV under column 8, lines 42 – 45.

Because the physiological pH of blood is 7.4, the production of nitric oxide, and iodine, as set forth by Trescony, et al., is negligible relative to requirements for anti-infective activity.

In the cited reaction of using iodide as a reducing agent for production of nitric oxide, column 6, line 5, iodine production is stoichiometrically half of nitric oxide on a mole equivalent basis. This, combined with data presented in Figure 4, shows that nitric oxide

release rates in PBS to be in the range of 50 and less picomoles per hour. Trescony, et al., does not specifically take into account that dilution volume of fluid bathing of its disclosure would further erode the effective concentration of product in the vicinity of the site from which it is formed. Trescony, et al., does not have data of the actual concentration achieved in blood which Trescony, et al., specifically notes greatly shortens the half-life of nitric oxide, further decreasing the average concentration of active product in the vicinity of his invention.

CONCLUSION

It is submitted that the present application is in form for allowance, and such action is respectfully requested.

The Commissioner is authorized to charge any additional fees which may be required, including petition fees and extension of time fees, to Deposit Account No. 08-1641 (Docket No. 25658-3202).

Respectfully submitted,

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CURRENT TOPIC

Nitric oxide and infectious diseases

David Burgner, Kirk Rockett, Dominic Kwiatkowski

Nitric oxide (NO) has undergone something of an image change in recent years. Previously considered a pollutant from car exhausts, NO has now been implicated in many physiological processes. Here we review the dual roles of NO in infection—as a critical agent of host defence but also as a central mediator of pathogenesis—using septic shock, bacterial meningitis, and malaria to illustrate some current concepts and controversies. We have limited our discussion of the important physiological functions of NO in the cardiovascular and neurological systems to instances where these impinge on its role in infectious diseases.

NO is formed by the oxidative deamination of the amino acid L-arginine by nitric oxide synthases (NOS) (fig 1). Three isoforms of this enzyme are described (table 1).[1] Neuronal NOS (nNOS or NOS1) is constitutively present in both the central and peripheral nervous systems, where NO acts as a neurotransmitter. Endothelial NOS (eNOS or NOS3) is constitutively expressed by endothelium and other cell types and is involved in cardiovascular homeostasis. In contrast, inducible NOS (iNOS or NOS2) is absent in resting cells, but the gene is rapidly expressed in response to stimuli such as proinflammatory cytokines. Once present, iNOS synthesises 100–1000 times more NO than the constitutive enzymes and does so for prolonged periods; the production of NO by eNOS and nNOS has been likened to a dripping tap, while that by iNOS to a fire hose. This high concentration of NO may inhibit a large variety of microbes, but may also potentially damage the host, thereby contributing to pathology.

Antimicrobial effects of NO

NO inhibits the growth of many bacteria and parasites in vitro.[2] The antimicrobial effect stems not from NO itself, but from reactive nitrogen intermediates formed by the oxidation of NO. For example, reaction between NO and the free radical superoxide (O_2^-) results in the formation of the unstable molecule peroxynitrite ($OONO^-$), while that between NO and thiol groups produces nitrosothiols. These reactive nitrogen intermediates inactivate key microbial enzymes, such as ribonucleotide reductase and aconitase, by reacting with iron containing groups in these enzymes. Studies have shown that murine macrophages can produce sufficient NO to kill leishmania parasites in vitro.[3] In vivo experiments using inhibitors of iNOS, and more recently knockouts of the iNOS gene, have clearly shown its role in the control of murine infections as diverse as malaria,[4] leishmaniasis,[5] tuberculosis,[6] and listeriosis.[7]

How much iNOS is produced by human leucocytes?

Although these observations undoubtedly indicate the potential importance of high concentration NO as an antimicrobial agent, a fundamental question remains as to the cellular

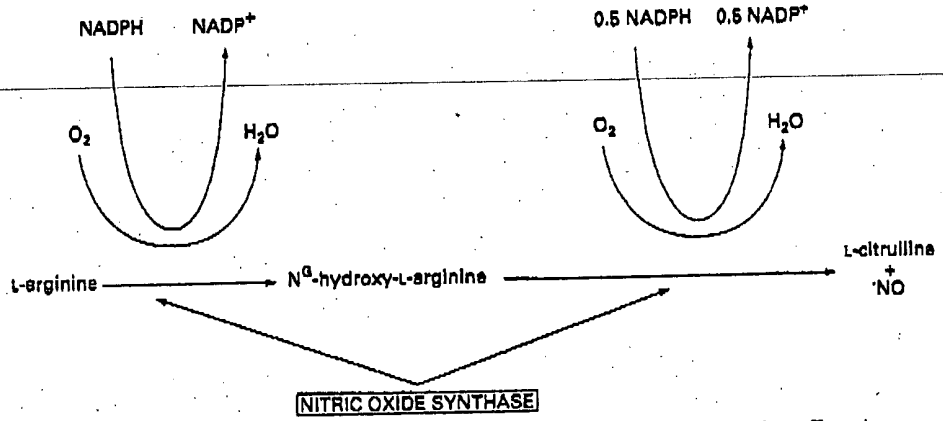

Figure 1 Conversion of L-arginine to L-citrulline plus nitric oxide as catalysed by nitric oxide synthases. Two primary steps have been identified. The first step, a two electron oxidation, is a hydroxylation of one of the guanidino nitrogens of L-arginine requiring molecular oxygen and nicotinamide adenine diphosphate (NADPH) to form Nω-hydroxy-L-arginine. The second step is a three electron oxidation, again requiring molecular oxygen and NADPH to perform an electron removal, oxygen insertion, and carbon-nitrogen bond scission to form L-citrulline and the free radical nitric oxide.

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Table 1 Some characteristics of human nitric oxide synthases (NOS) (enzyme classification EC 1.14.23)

Isoform	Alternative names	Molecular weight (kD) as monomer*	Intracellular location	Human cellular sources	Presence	Activation
nNOS	Neuronal NOS ncNOS NOS1 NOS type I	155	Membrane associated	Neurons, adrenal medullary, renal macula densa, glia, astrocytes,	Constitutive	Ca^{++} increase leading to calmodulin binding
iNOS	Inducible NOS NOS2 NOS type II	125–135	Cytosolic	Macrophages, monocytes, leukocytes, endothelium, smooth muscle, neutrophils, retinal pigmented epithelium, astrocytes, microglial, hepatocytes, Küpffer cells, fibroblasts, mesanglum	Inducible	Transcriptional induction Ca^{++} independent, calmodulin always bound
eNOS	Endothelial NOS ecNOS NOS3 NOS type III	135	Membrane (inactive) Cytosolic (active)	Endothelium, platelets, smooth muscle	Constitutive	Ca^{++} increase leading to calmodulin binding

*All enzymes active as dimers.

source of iNOS derived NO in people. In mice it is well established that NO is produced by macrophages, but in humans this evidence has proved more elusive. The human iNOS gene can be expressed in various cell types (table 1), but it has been much more difficult to demonstrate high levels of iNOS expression by leucocytes,[8] leading some to question the relevance of NO in human infection.[9] However, there is a growing view that this may reflect the greater complexity of iNOS regulation, and possibly a greater degree of stimulus and tissue specificity in humans compared to mice.[10] Whereas it is extremely difficult to stimulate leucocytes from healthy humans to produce iNOS in vitro, leucocytes from patients with inflammatory or infectious diseases have been shown both to express iNOS and to produce large amounts of NO. For example, NO producing leucocytes have been found in the peripheral blood of patients with septic shock[11] and malaria,[12] and in bronchoalveolar lavage fluid from patients with tuberculosis.[13] Thus the issue is not whether human leucocytes can make NO, but what stimuli are required and whether they do so in sufficient quantities to have a significant antimicrobial and pathological impact.

Investigating NO production in disease

Most studies rely on reactive nitrogen intermediates (in practice measurement of nitrite and nitrate (NO_3^-) in plasma or urine) as a surrogate for NO production. However NO_x concentrations are profoundly affected by diet, renal function, and hydration, all of which may be altered in severe illness.[12] This has made interpretation of studies difficult and yielded conflicting results, especially when patients selected as controls have conditions which also result in iNOS expression. In addition, systemic concentrations of NO_x may not reflect local production of NO in key organs, such as the spleen, bone marrow, or central nervous system. Measurements of other intermediates (such as nitrosothiols and nitrotyrosine, which reflect NO production and are not influenced by dietary nitrate), immunohistochemical investigation of iNOS expression, or investigation of genetic differences in the ability to produce NO may prove more useful in investigating the extent and site of NO production in inflammatory states.

NO and septic shock

Under physiological conditions eNOS derived NO activates soluble guanylate cyclase in vascular smooth muscle, increasing intracellular cyclic guanosine monophosphate and causing vasodilation. In sepsis, iNOS derived NO may mimic and exaggerate this process, causing chronic vasodilation, as well as interfering with and damaging other pathways. This makes iNOS an attractive candidate as a mediator of shock and multiorgan failure in sepsis. Animal models confirm widespread iNOS expression and NO production in response to Gram negative bacteria, endotoxin or inflammatory cytokines,[14] resulting in hypotension, organ failure, and death. These effects are prevented by non-selective NOS inhibitors, such as N^G-monomethyl-L-arginine (L-NMMA), which reverses systemic hypotension and improves survival in a number of animal models.[15] iNOS knockout mice, in which the iNOS gene has been experimentally disrupted, are protected from endotoxin induced hypotension in some studies,[7] although they are more susceptible to overwhelming infection with a number of intracellular organisms.[10]

There is good evidence for iNOS induction and increased NO production in human septic shock.[11] Initial reports of the use of non-selective NOS inhibitors (such as L-NMMA) in those with refractory sepsis induced hypotension showed an increase in mean arterial pressure and systemic and pulmonary vascular resistance.[16] Enthusiasm waned when it emerged that non-selective NOS inhibition actually increased mortality in septic patients, possibly by its negative effects on cardiac output and organ perfusion.[17] This discrepancy between animal and human studies highlights the need to extrapolate animal studies cautiously. Intraperitoneal or intravenous infusion of a known amount of endotoxin or bacteria obviously differs from human infection, where the inoculum and stage of infection are unknown. Many animal studies pretreat with NOS inhibitors, before bacterial challenge,

whereas in human trials pathological processes may be well advanced at presentation. More fundamentally, inhibition of a biological system with so many important physiological functions might be expected to have widespread and deleterious consequences. Current interest has focused on selective iNOS inhibitors, which might ameliorate the pathological effects of inappropriate NO production, but with less disruption of normal physiology. Although animal data support this approach,[16] a large trial of a selective iNOS inhibitor (546C88)[17] has produced disappointing results.

NO and meningitis

In bacterial meningitis, meningeal inflammation is initiated by local production of proinflammatory cytokines, especially tumour necrosis factor (TNF) and interleukin 1β,[19] which are both potent stimuli of iNOS expression in leucocytes, glia, and neurones. Cerebrospinal fluid NO_x concentrations are increased in experimental[20] and human meningitis, correlating with cerebrospinal fluid TNF concentrations in the latter.[21] Immunohistochemical staining of postmortem tissue from experimental animals[20] and humans (IA Clark, personal communication, 1999) show widespread iNOS expression and perimeningeal NO production. As before, NO may have a protective and pathological role; iNOS knockout mice are more susceptible to experimental *Listeria monocytogenes* infection,[7] and inhibition of NO worsens outcome in some models of bacterial meningitis, leading to focal cerebral ischaemia.[20] Conversely many of the pathological changes seen in bacterial meningitis, such as disruption of the blood-brain barrier, cerebral oedema, changes in cerebral blood flow,[22] and damage to cochlear cells,[23] can be prevented experimentally by NOS inhibitors. Dexamethasone reduces iNOS transcription in vitro and this might contribute to its protective effect in reducing cochlear damage and thus residual deafness. Whether NO acts as a neurotoxic or neuroprotective molecule may be dependent on its redox state; in its oxidised form (NO^+) inactivates glutamate receptors and reduces the neurotoxicity of excitatory amino acids, whereas the reduced form ($NO^•$) can form peroxynitrate, a potent neurotoxin.[24] The therapeutic use of NOS inhibitors in bacterial meningitis has been suggested, but as NO is likely to have profound effects on cerebral blood flow and other important physiological processes, our understanding of the role of NO in meningitis seems inadequate to justify this intervention at present.

NO and malaria

As in many parasitic infections, NO appears central to the host response in malaria, mediating the antiparasitic effects of proinflammatory cytokines at each stage of the parasite life cycle in both experimental and possibly human infection.[25] In addition to its protective role, NO has been suggested as a mediator in cerebral malaria. Cerebral malaria is the most infamous of the severe manifestations of *Plasmodium falciparum* infection, causing around 1 million deaths per year. The pathogenesis of cerebral malaria is debated, but derives from the sequestration of parasitised erythrocytes in the cerebral vasculature, where they may cause microvascular obstruction, local induction of inflammatory cytokines, or both. Clinically, there is often rapid recovery from profound coma without significant neurological sequelae, implying that a reversible process contributes to coma. It has been suggested that NO, produced by iNOS induced in vascular endothelium by proinflammatory cytokines, may cross the blood-brain barrier and affect local neuronal function, by mimicking and exaggerating the physiological effects of endogenous nNOS derived NO.[25] Investigating this hypothesis has proved difficult, as plasma concentrations of NO_x do not reflect local NO production within the brain. Some studies find that high concentrations of plasma NO_x correlate with depth and length of coma in cerebral malaria,[26] while others suggest that high plasma concentrations are protective against severe disease.[12] CSF NO_x, which may be more reflective of local cerebral production and is less affected by diet and other confounders, is increased in cerebral malaria.[27] Preliminary immunohistological studies suggest widespread iNOS induction in cerebral endothelium and resultant NO production (IA Clark, personal communication, 1999), although suitable control samples for such studies are difficult to obtain. Different genetic polymorphisms of the iNOS promoter region have been associated with increased risk of death from cerebral malaria in Gambian children[28] and with protection from severe malaria in Gabonese children,[29] suggesting that complex genetic factors may determine iNOS production and thereby influence clinical outcome.

Conclusions

The L-arginine NO pathway is a vital antimicrobial defence mechanism. Because of the ubiquitous nature of this pathway in normal physiology, it is easy to see how high and continuous NO production in sepsis might contribute to pathology, either by interfering with NO dependent pathways or by causing direct tissue injury. Our knowledge of the role of NO in sepsis is incomplete. The regulation of iNOS expression is complex and may be cell type, differentiation stage, and stimulus dependent, making the extrapolation of animal studies to the clinical setting difficult. As our understanding increases, modulation of the NO L-arginine pathway in infection may become a valuable therapeutic option.

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